Kinetics of quantitative HBsAg during antiviral therapy of chronic hepatitis B

A dissertation submitted in partial fulfillment of the requirements for

DM (Branch IV, Gastroenterology) examination of the

Tamil Nadu Dr. M.G.R. Medical University, Chennai, to be held in

August 2013.

Certificate

This is to certify that this dissertation entitled **"Kinetics of quantitative HBsAg during antiviral therapy of chronic hepatitis B"** is a bonafide work done by Dr. Gaurav Chawla in partial fulfillment of the rules and regulations for DM (Branch IV – Gastroenterology) examination of The Tamil Nadu Dr MGR Medical University, to be held in August 2013.

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INTRODUCTION

Chronic HBV infection is prevalent in Indian population with prevalence rate of 2-4% (1). It is well known to cause chronic hepatitis, chronic liver disease and hepatocellular carcinoma (HCC) (2). It is the most common cause of liver cirrhosis and hepatocellular carcinoma in most parts of Asia (3). It is a global health issue resulting in significant annual mortality worldwide. Studies from India have shown predominance of Genotype D and A (1,4,5,6). Patients with high viral counts and persistent inflammation (elevated liver enzymes or histopathologically) have more incidence of disease progression and related complication. Both e antigen and surface antigen are produced by Hepatitis B virus. These markers are useful in diagnosis and management of patients with hepatitis B .HBeAg seroconversion is important in natural history of CHB patients as it indicates decline in viral replication and subsequent remission of liver disease.20-30 % seroconverted patients may have reactivation of hepatitis B virus which may progress to E antigen negative chronic hepatitis. Such patients are still prone for development of chronic liver disease and HCC (7).

There are 4 phases in the natural history of chronic HBV infection: immunotolerant (IT), immunoclearance (IC), low replicative (LR) and E antigen negative hepatitis (ENH).These phases are defined by specific virological, biochemical and serological characteristics including liver enzmes,DNA loads, e Antigen status. These phases are not necessarily sequential and do not occurs in all individuals. Antiviral therapy including nucleos(t)ide inhibitors or interferon

therapy can lead to biochemical and virological remission. Recent evidence suggests that response to antiviral therapy is extrapolated into reduced incidence of chronic liver disease and hepatocellular carcinoma (8). Interferon therapy is effective only in 30-40% of patients and its use is limited by side effects and high costs (9). Nucleos(t)ide analogue therapy requires lifelong therapy with emergence of drug resistant mutant virus. Hence there is need for development of markers of response both before treatment and after treatment. The current gold standard for monitoring therapy in clinical settings is quantitative HBV DNA estimation by real time polymerase chain reaction (PCR) technique which has highest sensitivity and reproducibility. However quantitative HBV DNA estimation is costly and unavailable in some places.

Blumberg discovered HBsAg and since then it is used as hallmark of HBV infection. Initial tests for quantification of surface antigen were cumbersome, labour intensive and restricted to research laboratories. Moreover in 1980s there was no therapy available for HBV infection. Notably, advances have been made recently in the development of quantitative surface antigen assays which have allowed better viral replication monitoring. This interest in surface antigen quantification was derived from fact that it is found to be in good correlation with intra-hepatic covalently closed circular (ccc) DNA, the reservoir of HBV infection and template for HBV replication inside hepatocyte nuclei (10,11). Reduction in ccc DNA after antiviral therapy is

predictor of sustained viral response (12) .Changes in surface antigen levels before and after antiviral therapy correlate well with ccc DNA.

In recent years many studies have being conducted using surface antigen quantification for elucidating natural history of chronic hepatitis B and monitoring response to interferon or antiviral therapy. Surface antigen levels vary between different phases of CHB patients and also across different genotypes. Decline in surface antigen levels during and after interferon therapy has good predictive value for response to interferon therapy. Decline of 0.5 and 1.0 log surface antigen levels on pegylated interferon therapy predicts good respone to interferon therapy (13). HBsAg seroclearance represents the ultimate endpoint for chronic HBV infection as it is thought to represent the control of HBV replication.

Nonetheless, controversies exist in the use of surface antigen in clinical practice and it still remains a research tool confined to few institutions. There is paucity of data on the use of surface antigen quantification from Indian subcontinent where most patients are given nucleos(t)ide inhibitors. Predictive value of surface antigen quantification for response to nucleos(t)ide inhibitors is still low and requires further studies with longer follow up.

REVIEW OF LITERATURE

HBV DNA/Surface Antigen levels

Serological assays for surface antigen and HBV DNA measurements have improved in recent years due to technological advances which have immensely contributed in understanding the pathogen tic mechanisms of chronic hepatitis b infection. Several studies conducted independently showed consistent results between HBV DNA and surface antigen levels in different phases of chronic hepatitis B patients. To put into context, HBsAg production pathway and its relation with HBV DNA and intra-hepatic ccc DNA needs to be determined. There are three pathways for the production of HBsAg as depicted in Figure 1.

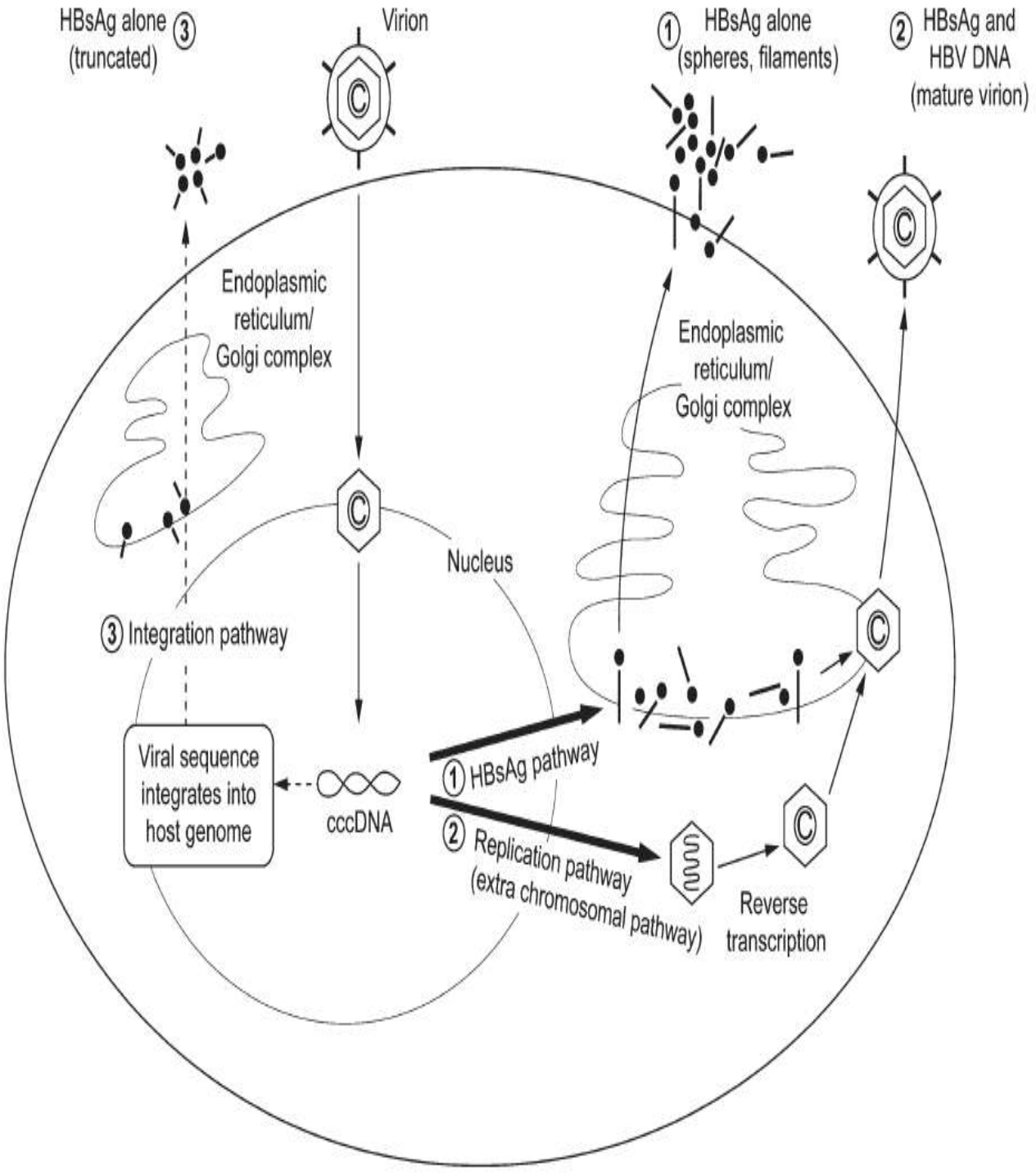

Fig. 1. Pathways of HBsAg production during HBV infection. Adapted from *J Hepatol* 2010;52:508-513 and HEPATOLOGY 2010;51:1933-1944.

Dane particle is the infectious, enveloped form of HBsAg. HBsAg also has non-infectious forms which are filamentous or spherical. These non infectious forms are produced in excess to the dane particle. Surface antigen (qHBsAg) correlated with ccc DNA in many studies (10,11,16,17).ccc DNA estimation requires complex techniques for estimation, though it is most accurate reflector of infected hepatocytes. Moreover it requires liver biopsy specimen analysis for ccc DNA estimation, therefore it has been used only in research settings.

HBV DNA quantification by PCR is required in diagnostic evaluation of all chronic hepatitis B patients. Serum HBV DNA decline reflects decrease in viral replication whereas surface antigen decline reflects reduction in translation of m RNA produced from transcriptionally active ccc DNA or integrated sequences. Thus, surface antigen quantification provides complementary information which may aid in characterisation of individual infection.

HBsAg / HBV DNA Levels-Phases of CHB

Previous cross-sectional studies that compared HBsAg and HBV DNA levels during various phases of chronic HBV infection revealed encouraging similar across different populations and genotypes. Although levels are variable during different phases of chronic hepatitis B, highest levels are observed in initial immunotolerant (IT) phase where alanine aminotransferases(ALT) levels are normal with no/minimal inflammation on liver biopsy. HBsAg levels declined in immune clearance(IC) phase and therafter it progressively decline in patients who achieved e antigen seroconversion with normal ALT levels (18). Lowest levels of surface antigen are observed in inactive carrier phase which also is characterised by highest HBsAg/HBV DNA ratio (14, 18, 19).

Chan et al followed 68 hepatitis E Antigen negative chronic hepatitis B patients and showed that decline trends of surface antigen (> 1 $\log_{10}$ IU/ml decline between initial and last visits) reflects better immune control over CHB infection, as objectively assessed by higher sero-clearance rates and stronger DNA suppression (18). Brunetto et al studied 209 genotype D patients and concluded that surface antigen levels are lower in inactive carriers than active carriers (20). Seto et al from Hong Kong studied 203 treatment naive e antigen negative CHB patients (genotype B and C) who achieved seroclearance. In this study optimal cut off to predict HBsAG seroclearance within 3 years of follow up were < 200 IU/ml(sensitivity 84.2%;specificity 73.4 %) and

0.5 log reduction IU/ml/year (sensitivity,62.8 % and specificity,88.7 %) (21).

HBeAg status	Immune Tolerance Phase	Immune Clearance Phase	Immune Control/ Inactive Carrier	Reactivated HBeAg-Negative Disease	Reference
	HBeAg-positive/ anti-HBe–negative	HBeAg-positive/ anti-HBe–negative	HBeAg-negative/ anti-HBe–positive	HBeAg-negative/ anti-HBe–positive	
1. Abbreviation: anti-HBe, antibody to hepatitis B e antigen.					
HBsAg level (log_{10}IU/mL)	5.0	3.0-4.0	1.5-2.2	2.5-3.0	18
	4.5	4.0	2.9	3.4	19
	5.0	4.4	3.1	4.0	14
HBV DNA level (log_{10}IU/mL)	7.5-8.5	6.0-7.0	1.0-2.4	3.9-4.6	18
	8.2	8.0	<2.6	5.0	19
	8.0	7.5	2.5	5.5	14
HBsAg/HBV DNA ratio (log IU/mL)	0.6-0.8	0.5-0.7	0.7	0.6-1.0	18
	0.5	0.5	1.0	0.6	19
	0.6	0.6	1.2	0.7	14

Levels of HBsAg and HBV DNA During the Natural Course of CHB and Their Relationship

Two recent studies from Asia followed up 390 and 103 e antigen negative CHB patients respectively, and found HBsAg level of < 100 IU/ml predictive of eventual HBsAg seroclearance (23, 24). Optimal cut off value of HBV DNA to define inactive carrier is still debated. In comparison to inactive carriers e antigen negative hepatitis (ENH) patients have higher DNA and surface antigen levels (14,18,19,20). Although exact values differ, surface antigen levels of 1 to 2 x 10^3 IU/ml and HBV DNA levels of 2 X 10^3 IU/ml have a diagnostic accuracy (94 to 100 %) for inactive carriers. However most studies are retrospective and require validation prospectively in patients infected with all genotypes. Further studies may confirm the utility of surface antigen in clinical management of CHB patients because they can be used to define more clearly who requires treatment and who do not. They may also reduce the need for liver biopsy in patients with mild elevation of liver enzymes and low levels of surface antigen and DNA levels (26).

qHBsAg trends during antiviral therapy

Entecavir (ETV)

Lee et al from Korea studied kinetics of qHBsAg in 95 CHB patients on entecavir (ETV 0.5 mg od) therapy for two years (27). Most patients were HBeAg + (60%) who were infected with genotype C . Baseline ALT (p=0.013), HBV DNA (p=0.040) and qHBsAg levels (p=0.033) were predictors of VR (virological response) in HBeAg + patients. The highest predictive value of VR was with baseline q HBsAg with the area under the curve of 0.823 ($P < 0.001$). The highest predictive value was obtained at a cut off value of 3.98 IU/mL with a sensitivity of 86.8% and a specificity of 78.9%.Serological respone (SR) which was defind as HBeAg loss at 24 months of therapy. In this subgroup of patients reduction in qHBeAg was more profound in patient who achieved SR (SR +). Correlation between qHBsAg and HBV DNA was most profound in E antigen positive patients and peaked after 6 months of ETV therapy.

Following conclusions can be made during this study.

1. Both quantitative surface antigen (qHBsAg) and quantitative E antigen decreased markedly with therapy.

2. Virological and Serological responses were predicted by baseline qHBsAg levels and on treatment decline in qHBeAg levels in HBeAg+ positive patients respectively.

Fung et al also studied patterns of surface antigen kinetics on entecavir therapy for two years (28).Among 166 CHB patients recruited in this study, 68 patients (41%) were e antigen positive. At baseline, qHBsAg levels correlated with age, HBV DNA and ALT levels ($r = -0.429$, 0.607, and 0.254, respectively, $P < 0.05$) in all patients.ETV therapy lead to reduced correlation between HBV DNA and qHBsAg levels and it was lost after 2 years of therapy. There was an overall decline in HBsAg levels from baseline to year 1 to year 2 (3,377.4 vs. 2,316.5 vs. 1,903.0 IU / ml, respectively, $P < 0.001$).14 patients have significant increase, 50 patients had significant decline and 102 patients had no significant changes after 2 years of ETV therapy .25 patients (37%) had E antigen seroconversion and 151 patients (91%) had HBV DNA suppression after 24 weeks of ETV therapy. Baseline qHBsAg or early decline in qHBsAg level at weeks 12/24 were not predictors of HBeAg seroconversion at 24 weeks.

Conclusions derived from this study were

1. ETV therapy lead to profound decline in HBV DNA levels with no significant decline in qHBsAg levels.
2. There was no correlation between q HBsAg decline at week 12/24 with HBV DNA suppression or E antigen seroconversion.

Though there were some limitations noted in this study. Firstly, patients were followed up for only short span of time. Secondly, none of the patient had HBsAg seroclearance so predictors of response to

HBsAg seroclearance could not be calculated. Thirdly, genotypic analysis, ccc DNA estimation and resistant mutational analysis were not done.

Jung et al from Korea studied 28 CHB patients who were E antigen positive on ETV therapy (29). qHBsAg level showed a mean of 4.0, 3.7, and 3.6 log10IU/mL at baseline, 6, and 12 months, respectively.HBV DNA levels showed a mean of 8.1, 3.1, and 2.4 log10 copies/mL at baseline, 6, and 12 months, respectively. Decline in q HBsAg and HBV DNA were significant ($P<0.001$).After 12 months of therapy, there was correlation noted between HBV DNA levels and qHBsAg levels. ($g=0.391$, $P=0.044$).HBsAg response was noted in five patients and cumulative incidence of HBeAg loss was noted in 80% as compared to 30 % in those without HBeAg loss($p=0.034$). This study showed that decline in surface antigen level correlated with HBV DNA on entecavir therapy and significant decline in surface antigen at 1 year may be predictor of antiviral response, particularly HBeAg seroconversion. No genotypic analysis was done in this study but results may be applicable to Genotype C patients as it is the most prevalent genotype in Korea.

Tenofovir

HBsAg seroclearance was noted after tenofovir therapy in e antigen positive patients only (30).After 1,2 and 3 years of tenofovir therapy, seroclearance rates of 3%,6% and 8% were noted. Gane et al studied 176 HBeAg + CHB patients on tenofovir (30). He observed median baseline qHBsAg level 4.56 log10IU/mL. The median qHBsAg change from baseline in this study at W144 was -0.63log10IU/mL. HBsAg loss was noted in 8 % of HBeAg positive patients in each group.In this study, 6% and 7% seroconverted to anti-HBs by W144 (TDF-TDF and ADV-TDF groups, respectively).Predictors of HBsAg loss noted in this study were: higher baseline qHBsAg level(OR= 48.8), higher baseline necro-inflammatory score(OR=1.7) and patients with Genotype D/A(OR =10.4).

Telbivudine (TDT)

Wursthorn et al studied 162 HBeAg + CHB patients on telbivudine therapy for three years (31).qHBsAg levels and its correlation with HBV DNA was studied. qHBsAg levels (mean ± SD) reduced from baseline (3.8 ± 0.6 $\log_{10}$IU/mL) to treatment week 24 (3.4 ± 0.7 $\log_{10}$ IU/mL), treatment year 1 (3.3 ± 0.8 $\log_{10}$ IU/mL), and treatment year 3 (3.0 ± 1.4 $\log_{10}$ IU/mL) (P <0.0001).HBsAg seroclearance was noted in 6% after three years of therapy.8 of 32 patients with rapid HBsAg decline versus none of 56 patients with steady HBsAg levels achieved HBsAg loss at year 3 (P = 0.0024).Fastest decline of surface antigen was noticed in genotype A patients, implying genotypic analysis as a determinant of surface antigen decline. Liver biopsy performed after 1 year in those who achieved sero-clearance revealed absence of viral antigens. It can be concluded from this study that effective suppression of viral replication as measured by rapid qHBsAg decline on TDT therapy could predict HBsAg seroclearance rates.

Wei Cai et al studied 17 HBeAg + patients treated with TdT for two years (32). HBsAg levels < 2log10 IU/ml at 2 years were highly predictive of sustained viral response at 2 years off-treatment(PPV of 93 % and negative predictive value(NPV) of 100 %).In this subgroup of patients surface antigen consistently declined from baseline. qHBsAg decline rates of > 0.8 and > 1 log 10 IU/ml at 6month and 1 year were predictive of sustained response (PPV-75 %,NPV-86% at week 24; PPV-75 % and NPV86% at week 52).

Interferon (IFN) therapy

Janssen et al first proposed in 1994 that surface antigen quantification may be useful for monitoring interferon treated HBeAg positive patients when a significant decline occurred in interferon treated patients who had HBeAg seroconversion responded to therapy but not in patients without HBeAg seroconversion($p<0.001$) (33). Thus HBsAg quantification was proposed as a simple method for monitoring therapy in CHB patients but lack of commercial assays precluded its widespread utility until recently.

Manesis et al and Wigand et al proposed that qHBsAg monitoring could predict HBsAg sero-clearance after 5.4 years of SVR to IFN therapy or after 10.6 years of lamivudine (LAM) therapy (34, 35). Subsequently several other studies have clearly demonstrated that HBsAG decline is more prominent and rapid with IFN therapy than with nucleos(t)ide analogues(30,31,36-41).It could be deduced that qHBsAg decline is immune modulated rather than by antiviral therapy.

Predicting Response to IFN therapy.

Since response rate of pegylated interferon therapy in HBeAg + and HBeAg – patients is 30 % and 20 % respectively, it is important to derive markers predicting treatment success both for patient and physician. Chan HL showed that higher response to IFN therapy in HBeAg + patients whose baseline qHBsAg levels were <10 000 IU/ml (42). Though this result was not validated in other studies, significant on treatment levels correlated with sustained response to interferon therapy. 202 e antigen positive patients in Europe were studied by Sonneveld et al,who were treated with IFN for 52 weeks. qHBsAg decline at week 52(3.3 versus 0.7 log 10 IU/ml) and week 78(3.4 versus 0.35 log 10 IU/ml) predicted sustained response (37)

Treatment with PEG-IFN alpha 2b with or without lamivudine (LAM) in 92 E antigen positive patients from Hong Kong for 32 to 48 weeks was studied by Chan et al. They demonstrated qHBsAg level < 1500 IU/ml at week 12 and qHBsAg levels < 300 IU /ml at week 24 could predict a SVR 12 months after treatment (42).

Piratvisuth et al also demonstrated that qHBsAg level <1500 IU /ml at month 3 of interferon therapy was associated with HBeAg conversion rate of 57 % at 6 months after treatment. Among patients who seroconverted 18 % had HBsAg seroclearance(44).

In HBeAg- patients, studies have demonstrated that baseline surface antigen levels do not predict response to peg-IFN therapy but sustained responders have mark decrease in surface antigen levels at week 48 (2.1 + 1.2 log 10 IU/ml) . Brunetto et al showed that both qHBsAg level <10 IU /m(12% of patients)l at end of treatment and >1.2 log 10 IU/ml decline during therapy were associated with surface antigen clearance 3 years after therapy (RR of 22.8 and 10.8 respectively, p < 0.0001) (40).Moucari et al showed similar results with 0.5 and 1 log decline in qHBsAg levels at week 12(19% of patients) and 24(23 % of patients) of PEG-IFN alpha 2a,had good predictive value for sustained response(89 % at month 3 and 92% at month 6) (45). Marcellin et al studied 120 e antigen negative patients and showed that decline of > 10% at 12 week of interferon therapy was associated with a 1 year off therapy SVR of 47 % and seroclearance rate of 23 % at 5 years after therapy (41). HBV DNA declines throughout the treatment and there was no difference between responders and relapsers.

Surface antigen levels predicting response to interferon therapy were noticed across all genotypes (46). Although these low surface antigen levels, predicting response to therapy were achieved in less than 25 % of patients, it can motivate or encourage interferon treated patients to complete therapy.

Predicting Non-Response

Surface antigen quantification may predict non-response to therapy and identify patients who need early discontinuation of therapy or alternative treatment regimes. Both e antigen positive or negative patients have demonstrated high negative predictive value (NPV) for response. Sonneveld et al demonstrated NPV of 97 % in 202 e antigen positive patients (74 % were genotype A or D) on interferon therapy based on any decline in surface antigen levels at week 12 (37).Piratvisuth et al reported 82 % NPV for response in e antigen positive genotype B/C infected patients having surface antigen decline at week 12 (44).ChanHL et al from Hong Kong reported NPV of 86 % for surface antigen levels < 1500 at 12 weeks and 89 % for surface antigen levels < 300 at 24 weeks in e antigen positive patients (42). Another Chinese study of e antigen positive infected patients showed that surface antigen levels < 1500 at 3 months and < 2840 at 6 months had NPV of 91 % and 95 % respectively for response. For e antigen negative patients Moucari et al reported a NPV of 90% for > 0.5 log decline at week 12 and 97% for > 1 log decline at week 24 in a study population involving all genotypes (45). Rijckborst et al studied genotype D e antigen negative patients and demonstrated NPV of 100 % based on combination of decline in surface antigen level and > 2 log10 IU /ml decline in HBV DNA levels from baseline to week 12 (47).This result was validated in another cohort of patients so stopping rule for interferon therapy can be applied with 100 % NPV. This can help in patient management and encourage patients to consider interferon

therapy as first line treatment. This is particular applicable in e antigen negative patients requiring lifelong nucleos(t)ide therapy.

Special Populations

1. HBV/HCV coinfection

Surface antigen kinetics was done in HBV/HCV co-infected patients who were treated with INF and Ribavirin for HCV infection. Interestingly, 50 % surface antigen decline at week 12 in patients who had undetectable HBV DNA at baseline had reduced likelihood of HBV DNA reactivation (PPV-89.5 %) (48).In this study low surface antigen levels were associated with HBsAg sroclearance (40% for baseline HBsAg levels < 20 IU/mL and 2% for levels >120 IU/mL, P < 0.05).This study raises the possibility that identification of those patients who are at risk of reactivation and needs NA therapy may be possible.

2. HBV/HIV coinfection

Zoutendijk et al studied 104 HIV/HBV infected patients (predominantly genotype A) in Netherlands who were treated with tenofovir as part of HAART therapy and showed that early decline of surface antigen within 1 year correlated with improved CD 4 counts and HBsAG seroclearance in e antigen positive patients (49). This explains the importance of immune restoration by HAART in HIV/HBV co-infected patients. A significant decline in HBsAg level of 2.2log IU/mL at year 6 was noted within the HBeAg-positive population, while a minimal decline was observed in HBeAg-negative patients. A decline in HBsAg

of at least 2log IU/mL at 24 weeks was highly predictive of HBsAg seroclearance.

Perspectives

There is still more to learn about decline of surface antigen kinetics on therapy. Prediction of seroclearance/seroconversion by surface antigen quantification needs further confirmation. Majority studies are done in Europe and Asia and we may need studies in different parts of world to show its utility across all genotypes. Prediction models using HBV DNA, ALT and surface antigen needs to be determined. Nevertheless, with surface antigen quantification we may be able to individualise antiviral therapy in some patients of CHB.

AIMS

Primary Objective

To study the pattern of decline in Hepatitis B surface antigen levels (qHBsAg) on anti-viral therapy (both nucleos(t)ide and interferon therapy) and its correlation with decline in HBV DNA levels.

Secondary Objectives

1. Study the predictors of virological response.
2. Study the correlation of qHBsAg decline with e antigen status, ALT.

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METHODOLOGY

Study Population

This is a prospective study which was conducted in department of Hepatology at Christian Medical College, Vellore from Jan 2011 to December 2012.Patients with chronic hepatitis B(CHB) who were started on antiviral therapy during this period were included in the study. Antiviral therapy (nucleos(t)ide or interferon) was started according to clinician's discretion. The protocol followed was:

a. e antigen positive (HBeAg +ve) patients with DNA loads >20 000 IU/ml irrespective of ALT levels

b. e antigen negative (HBeAg –ve) with DNA loads > 2000 IU/ml and elevated ALT levels

c. HBeAg –ve patients with DNA loads > 20,000 IU/mL.

c. Presence of chronic liver disease irrespective of DNA levels

Nucleos(t)ide analogues that were given to patients included tenofovir (300 mg once daily), entecavir (0.5 mg once daily) or lamivudine (100 mg once daily)/adefovir(5 mg once daily).HBeAg + patients were given either peginterferon alpha 2 a/b or conventional interferon alpha 5 milion s/c units thrice weekly upto 6 months. Our department policy was to offer interferon therapy for e antigen positive patients. e antigen negative patients were not considered for interferon therapy due to low success rates in this group.

- **Inclusion Criteria**

❖ Chronic hepatitis B patients initiated on antiviral therapy(including cirrhosis)

- **Exclusion Criteria**

❖ Acute hepatitis B

❖ Co- infection with hepatitis C and HIV

❖ Prior antiviral therapy

Institutional research committee approved the study and it was conducted in accordance with declaration of Helsinki. Written informed consent was taken from participants, prior to enrolment in study. All patients underwent clinical examination and blood tests including liver function tests, prothombin time with INR, alpha fetoprotein, HBsAg, E antigen, quantitative HBV DNA PCR, qHBsAg and ultrasound examination on initial evaluation. IgM core was done when evidence of chronicity was not clear. Thereafter patients underwent quantitative HBV DNA PCR, qHBsAg, ALT on follow up visits at 3 to 6 months,12 and 18 months. We wanted to have atleast 2 follow up visits .Maximum follow up of patients was at 18 months. Christian Medical College fluid research grant was obtained for study.

Definitions

Chronic Hepatitis B: All patients with HBsAg positivity for more than 6 months or patients with HBsAg positivity with negative IgM core.

Cirrhosis of liver: All patients with clinical, biochemical (low albumin and elevated PT with INR) evidence of CLD confirmed by either radiological (ultrasound showing shrunken liver with hepatic nodules or portal vein >13mm) or endoscopic evidence of portal hypertension or histology

Virological response (VR): Patients with ≥ 2 log decline in HBV DNA at 24 weeks.

Complete Virological response (CVR): Patients with undetectable (nil or < 10) HBV DNA levels at 24 weeks.

NonResponse(NR): Patients with < 1 log reduction in DNA levels at 24 weeks.

Laboratory tests:

Routine blood tests including serum alanine aminotransferase (ALT), albumin, bilirubin and creatinine levels were measured using automated analyser.

Serology testing

HBsAg quantification was performed using the Architect chemiluminescent system (Abbott, Weisbaden, Germany).The dynamic range of the assay is between 0.05 to 250 IU/mL. Samples with >250

IU/mL were diluted at 1:20 and 1:500 with the Architect HBsAg diluent and tested. Samples with >125,000 IU/mL were further tested with 1:999 dilution.

HBeAg and anti-HBc testing was performed in an enzyme immunoassay (Diasorin S.P.A., Saluggia, Italy) according to the manufacturers instruction. The upper limit detection (ULD) of assay after the recommended dilution is 250000 IU/mL.

HBV DNA quantification

DNA was isolated from plasma sample using the automated m2000sp system (Abbott, Weisbaden, Germany). The isolated DNA was then quantified using the m2000rt system (Abbott, Weisbaden, Germany).Lowest limit of detection is 10 IU/ml. All laboratory assays were done every 6 months

Statistical Analysis

Serum surface antigen and HBV DNA levels were logarithmically transformed for analysis. Continuous variables are presented as mean (±SD) or median (inter-quartile range [IQR]), where appropriate Continuous variables were compared using the t test or the Mann-Whitney U test and categorical variables were compared using the χ^2 or Fisher exact test. Spearman's correlation coefficient(r) was used to derive correlation between two variables.

Statistical analyses were performed using SPSS, version 20.0 (SPSS, Chicago, IL). All statistical tests were 2-sided and p value of >0.05 was taken significant.

RESULTS

Total of 88 patients were started on antiviral therapy between Jan 2011 and December 2012. Fifty five patients had follow up at 6 months, twenty eight patients had follow up at 1 year and 11 patients had follow up at 18 months. A total of 181 samples were analysed

Baseline clinical and laboratory parameters are depicted in table 1. Majority of patients were from Eastern India (48.86%). Forty eight patients were HBeAg –ve (54.54%) and forty patients were HBeAg +ve (45.46%). Majority (n=78) were males with median age of 36.50 (7 -69) years. Median baseline AST and ALT were 39 (9-1204) and 41 (8-1518).Median baseline qHBsAg and HBV DNA levels were $\log_{10}$ 3.98 (0.19 – 5.40) IU /mL and $\log_{10}$ 5.39(1.15 -9.90) IU/mL respectively. In HBeAg + ve patients, baseline qHBsAg and HBV DNA levels were $\log_{10}$ 4.35(2.89 -5.40) IU/mL and $\log_{10}$ 6.70(2.62-9.90) IU/mL respectively and in HBeAg -ve patients they were $\log_{10}$ 3.711 (0.19 - 5.07) IU/mL and $\log_{10}$ 4.00 (1.15 - 9.00) IU/mL respectively. Baseline HBV DNA and qHBsAg levels were significantly higher in e antigen positive patients as compared to e antigen negative cases ($P < 0.0001$). Median duration of follow up of study population was 12 months (6 to 18 months).

Among baseline characteristics following parameters correlated with qHBsAg

a. DNA: ($r=0.487, p < 0.01$)

There was no correlation of ALT with baseline qHBsAg(r=0.118, p =0.275).

Table 1-Comparison of Baseline Characteristics between e antigen positive and negative patients

Characteristics	TOTAL (N=88)	e ANTIGEN + (N=40)	e ANTIGEN – (N=48)	P value
Age (years) Median(range)	36.50 (7 - 69)	30 (7 - 63)	41 (19 - 69)	0.0001
Males(%)	78 (88.6)	34 (85%)	44 (91.67)	
Ethnicity(%) Eastern India North East South India Others	43 (48.86) 12 (13.64) 22 (25) 10 (11.36)			
AST Median(range)	39 (9-1204)	43.50 (16-96)	33 (9-1204)	0.022
ALT Median(range)	41 (8-1518)	46 (8-889)	31 (10-1518)	0.001
DNA $\log_{10}$ IU/Ml	5.39 (1.15 -9.90)	6.70(2.62-9.90)	4.00(1.15 - 9.00)	0.0001
qHBsAg $\log_{10}$ IU/mL	3.98 (0.19 -5.40)	4.35(2.89 -5.40)	3.711 (0.19-5.07)	0.0001

THERAPY(%) Tenofovir(TDF) Entecavir(ETV) Lamivudine/+Adefovir Interferon(IFN)	 39(44.32) 33(37.5) 8 (9.09) 8 (9.09)	 18(45) 12(30) 2 (5) 8 (20)	 21 (43.75) 21 (43.75) 6 (12.5) 0 (0)	
CIRRHOSIS(%)	32 (36.36)	13(32.5)	19(39.58)	

Correlation between HBV DNA and Surface antigen levels

There was correlation noted between HBV DNA and qHBsAg levels at baseline and at 24 weeks of therapy in e antigen positive cases as shown in Table 2. Baseline qHBsAg significantly correlated with HBV DNA levels with a correlation coefficient(r) of 0.487 ($p < 0.01$).When a subgroup analysis was noted in e antigen positive and negative patients significant correlation between these two tests was also noted in e antigen positive patients($r = 0.560$, $p < 0.01$) as opposed to e antigen negative patients($r=0.256$).Correlation between surface antigen and HBV DNA was maintained after 6 months of antiviral therapy ($r=0.344$,$p < 0.05$) in e antigen positive patients. There was no correlation noted at 48 weeks($r = 0.359$, $p=0.08$)in e antigen positive and negative patients($r=0.425$,$p=0.168$).In HBeAg -ve cases there was excellent correlation noted after 18 months of therapy($r=0.894$, $p=0.05$)

Table 2: Correlation between HBV DNA and qHBsAg

	All patients		E antigen positive		E antigen negative	
	rho	p value	rho	p value	rho	p value
24 weeks	0.344	0.05	0.427	0.05	0.146	0.433
48 weeks	0.359	.085	0.014	0.966	0.425	0.168
72 weeks	0.426	.252	0.600	0.4	0.894	0.05

Correlation of qHBsAg with HBV DNA levels with type of antiviral therapy.

Majority were started on tenofovir (44.37%) followed by entecavir (37.5%) and lamivudine/+ adefovir (9.09 %) or interferon therapy (9.09%). Compliance to antiviral therapy was ensured on every follow up. No adverse drug effects leading to discontinuation of antiviral therapy were noted on follow up. None of the patients had deranged renal parameters on tenofovir therapy. Baseline correlation between HBV DNA and qHBsAg levels were noted on entecavir (ETV) and tenofovir (TDF) therapy with correlation coefficient (r) of 0.428 ($p < 0.005$) and 0.567 ($p < 0.001$)(See table 3).Correlation was maintained on entecavir therapy at 6 months but not on tenofovir or interferon

therapy. There was no correlation after one year of therapy with either entecavir or tenofovir therapy. There was significant correlation between these tests at 1 year on interferon therapy.

Median qHBsAg and HBV DNA at baseline, 24 weeks, 48 weeks and 72 weeks on ETV therapy was $\log_{10}$ 3.73 and $\log_{10}$ 5.95, $\log_{10}$ 3.79 and $\log_{10}$ 1.65, $\log_{10}$ 3.46 and $\log_{10}$ 1.45 and $\log_{10}$ 3.11 and $\log_{10}$ 1.00 respectively.

Median qHBsAg and HBV DNA at baseline, 24 weeks, 48 weeks and 72 weeks on TDF therapy was $\log_{10}$ 4.16 and $\log_{10}$ 5.30, $\log_{10}$ 4.29 and $\log_{10}$ 1.72 , $\log_{10}$ 4.06 and $\log_{10}$ 1.23 and $\log_{10}$ 4.15 and $\log_{10}$ 1.32 respectively

Table 3 Correlation with Antiviral therapy

Duration	**ETV**		**TDF**		**IFN**	
	rho	p value	rho	p value	rho	p value
24 weeks	0.496	<0.05	0.226	0.277	0.80	0.104
48 weeks	0.559	0.117	0.190	0.577	0.962	< 0.005
72 weeks			0.880	0.220		

qHBsAg and HBV DNA Decline with therapy

There was no significant difference in qHBsAg at various time points during this study. (See Table 4)

Table 4: qHBsAg and HBV DNA levels

	Baseline	24weeks	48weeks	72 weeks	P value
qHBsAg (log_{10} IU/ml)	3.98 (0.19 to 5.40)	4.24 (0.19 to 5.38)	3.823 (0.26 to 4.98)	3.82(2.98 to 4.58)	0.419
HBV DNA(log_{10} IU/ml)	5.39(1.15 to 9.90)	1.67(1.00 to 7.70)	1.59 (1.00 to 6.00)	1.23(1.00 to 7.00)	<0.05
qHBsAg (HbeAg+) (log_{10} IU/ml)	4.34(2.89 to 5.40)	4.36(2.09 to 5.380)	4.16 (0.26 to 4.98)	3.96 (3.30 to 4.23)	0.552
(HBeAg -)	3.71(0.19 to 5.07)	3.61(0.19 to 4.92)	3.34(0.41 to 4.65)	3.24 (2.98 to 4.58)	0.421

It changed from log_{10} 3.98 (0.19 to 5.40) at baseline to log_{10} 4.24 (0.19 to 5.38) at 6 months .It declined to log_{10} 3.823 (0.26 to 4.98) at 12 months and remained stable at log_{10} 3.82(2.98 to 4.58) at 18 months.

HBV DNA significantly declined from log 5.39(1.15 to 9.90) to log_{10} 1.67(1.00 to 7.70) ($p < 0.05$) at 6 months. It was log_{10} 1.59 (1.00 to 6.00) at 12 months and the lowest at 18months 1.23(1.00 to 7.00) (See Fig 1).

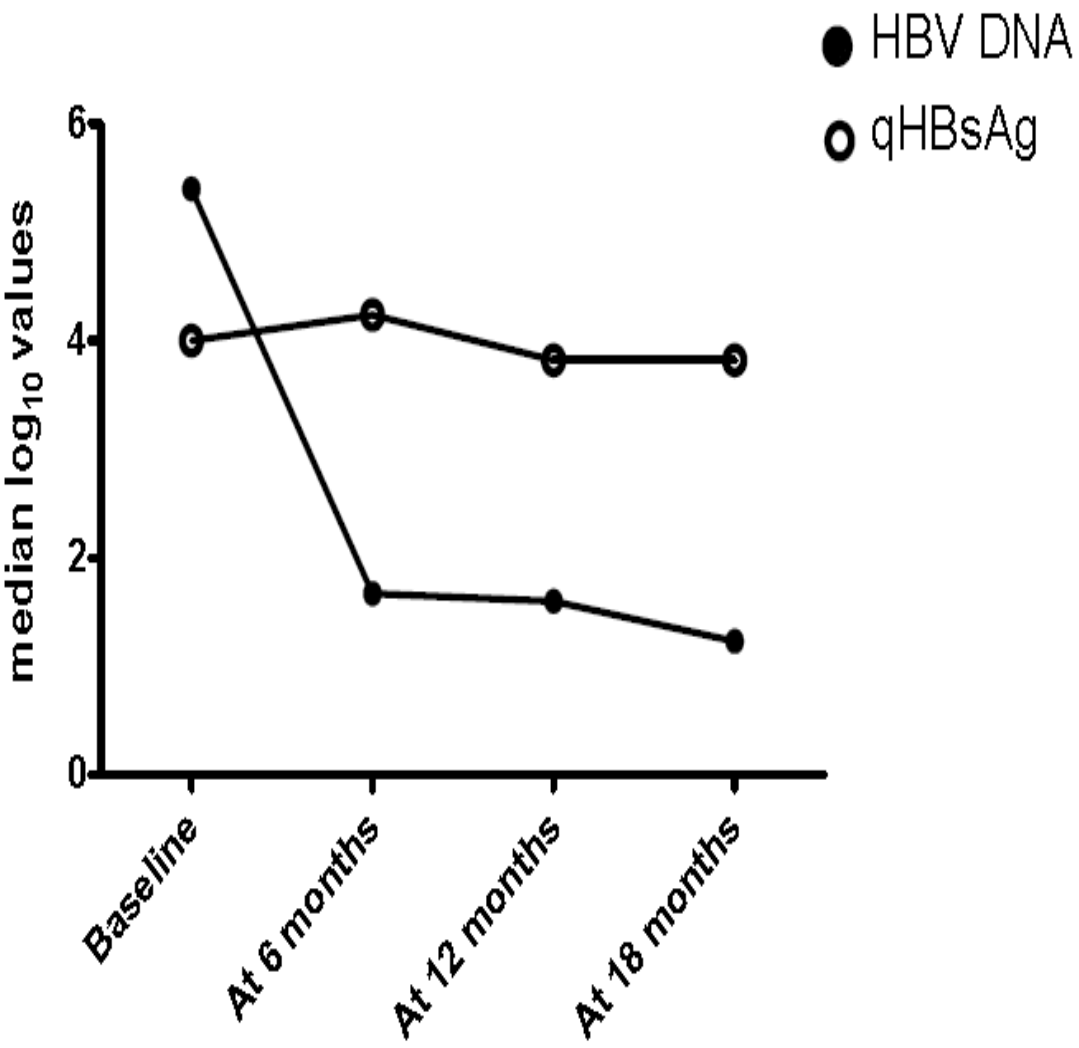

Fig -1 Median HBV DNA and qHBsAg levels

HBeAg +ve

In e antigen positive patients qHBsAg changed from $\log_{10}$ 4.34(2.89 to 5.40) to $\log_{10}$ 4.36(2.09 to 5.380) at 6 months. It then decreased to $\log_{10}$ 4.1645 (0.26 to 4.98) at 12 months and lowest at 18 months $\log_{10}$ 3.96 (3.30 to 4.23). In this subgroup of patients log HBV DNA decreased significantly from $\log_{10}$ 6.70(2.62 to 9.90) at baseline to $\log_{10}$ 3.00(1 to 7.70) at 6 months ($p< 0.05$). It further reduced to $\log_{10}$ 2.38(1.00 to 6.00) at 12 months .It changed to $\log_{10}$ 3.30(1.00 to 7.00) at 18 months. (see fig 2).

HBeAg -ve

In e antigen negative patients log qHBsAg declined from baseline $\log_{10}$ 3.71(0.19 to 5.07) to $\log_{10}$ 3.61(0.19 to 4.92) at 6 months. It then stabilised to $\log_{10}$ 3.34(0.41 to 4.65) at 12 month and $\log_{10}$ 3.24 (2.98 to 4.58) at 18 months. In e antigen negative patients HBV DNA declined from $\log_{10}$ 4.00 (1.15 to 9.00) to 1.10(1.00 to 6.00) at 6 months. It stabilised to $\log_{10}$ 1.04(1.00 to 4.90) at 12 month and $\log_{10}$ 1.00(1.00 to 1.41) at 18 months.(fig3) Overall DNA decline was significantly different at all time points in e antigen positive and negative patients($p< 0.05$) as compared to qHBsAg levels.

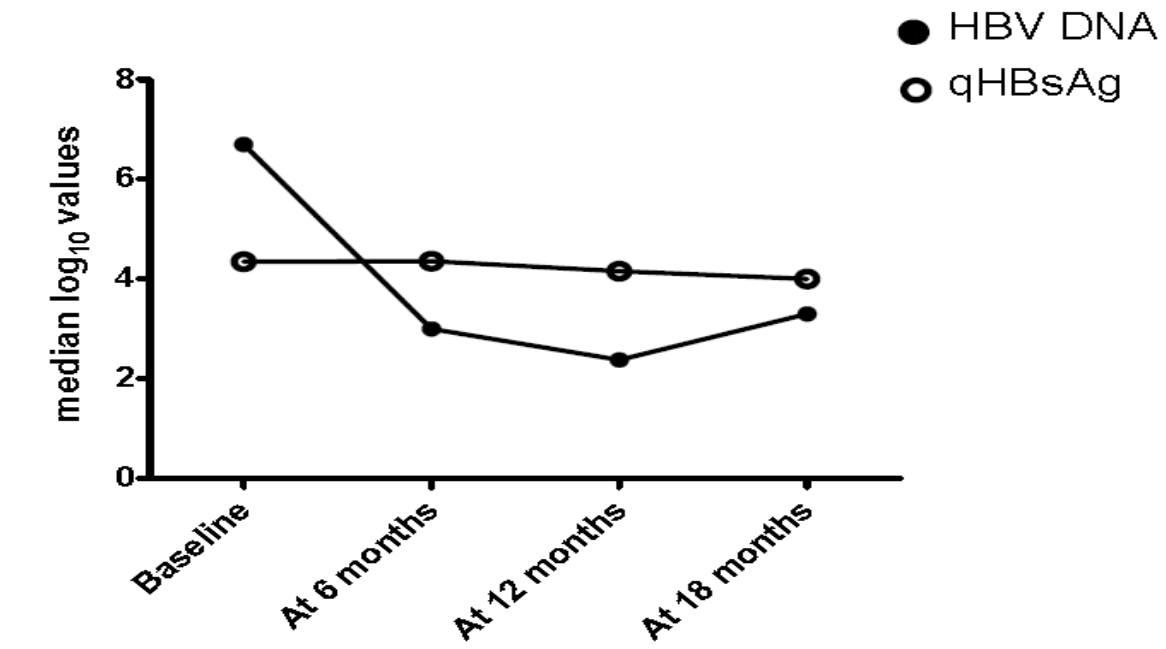

Fig 2 Median HBV DNA and qHBsAg levels in HBeAg +ve patients

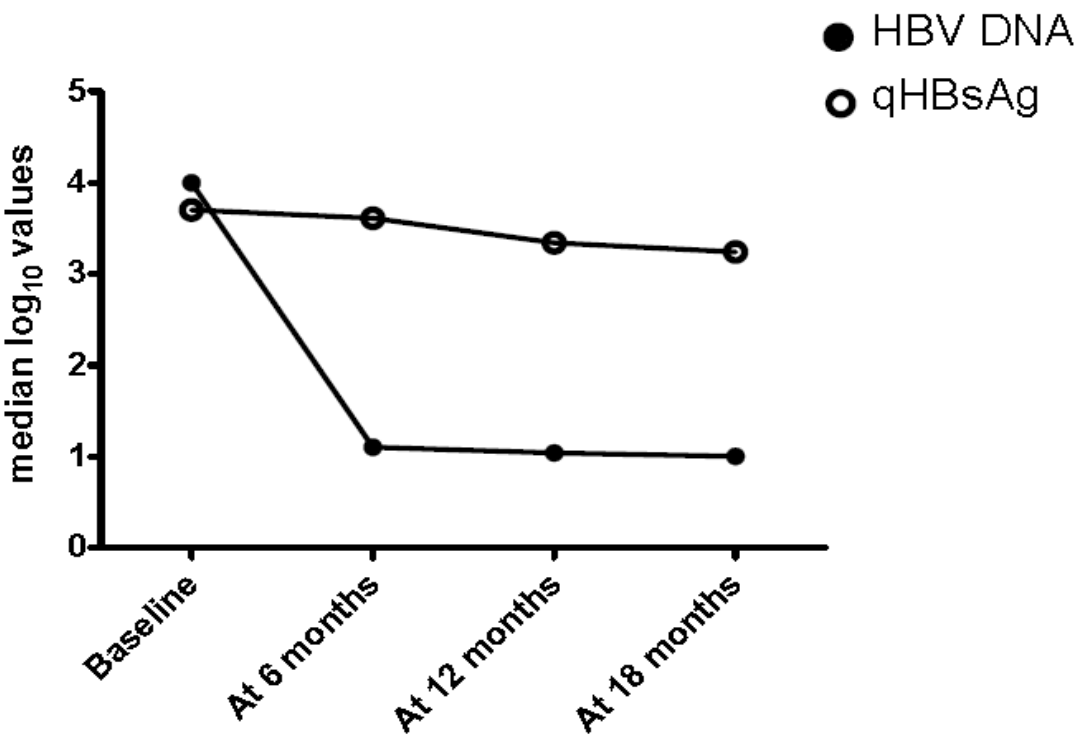

Fig 3 Median HBV DNA and qHBsAg levels in HBeAg -ve patients

Virological response (VR):

72.7 % (n=40) patients achieved virological response. Univariate analysis showed that that baseline qHBsAg, HBV DNA, ALT, age or sex were not predictors of virological response. qHBsAg and HBV DNA at baseline in those who achieved VR were $\log_{10}$ 5.85 (1.63 to 9.90) and $\log_{10}$ 4.08 (0.19 to 5.40)IU/mL .In HBeAg + ve VR patients, it was $\log_{10}$ 4.64 (3.52 to 5.40) and $\log_{10}$ 7.66(4.78 to 9.90)IU/mL respectively and in HBeAg –ve VR patients , it was $\log_{10}$ 3.64 (0.19 to 4.78) and $\log_{10}$ 4.30 (1.15 to 7.30)IU/mL .In e antigen positive patients only HBV DNA was predictor of virological response($P < 0.05$).

Complete virological response (CVR):

Among patients who achieved virological response,CVR was noted in 18 patients (2 in HBeAg +ve and 16 in HbeAg – ve patients).On univariate analysis, baseline ALT and log HBV DNA were predictors of CVR ($p < 0.05$) . qHBsAg was not a predictor of CVR($p = 0.057$). On multivariate analysis only baseline HBV DNA was significant predictor of CVR.AUC for predicting CVR was lowest for qHBsAg at 0.659, followed by ALT at 0.721 and highest for HBV DNA at 0.819(see Fig 5).Median baseline qHBsAg and HBV DNA in those who achieved CVR were $\log_{10}$ 3.72(0.19 to 5.06) and $\log_{10}$ 3.77(1.15 to 6.30) IU/mL respectively. Difference in qHBsAg(Δ qHBsAg) at 24 weeks was $\log_{10}$

0.048 IU/ml. In HBeAg + patients, median baseline qHBsAg and HBV DNA were $\log_{10}$ 4.40(3.31 to 5.40) and 6.60(3.30 to 9.90) IU/mL respectively. Δ qHBsAg in this group was 0.23. In HBeAg – ve patients median baseline qHBsAg and HBV DNA were $\log_{10}$ 3.64(0.19 to 4.53) and $\log_{10}$ 3.70(1.15-6.30) IU/mL respectively. Δ qHBsAg in this group was 0.042.

Non Response:

13 patients were non responders (23.21%).Eight patients were e antigen negative and five patients were e antigen positive. Univariate analysis showed that that baseline qHBsAg, HBV DNA or ALT were not predictors of non response. qHBsAg and HBV DNA at baseline in those who achieved VR were $\log_{10}$ 3.49(0.19 to5.40) and $\log_{10}$ 4.60(1.15 to 7.78)IU/mL. Among this subgroup of patients, five were on TDF and IFN each , two patients received LAM and one on ETV therapy.

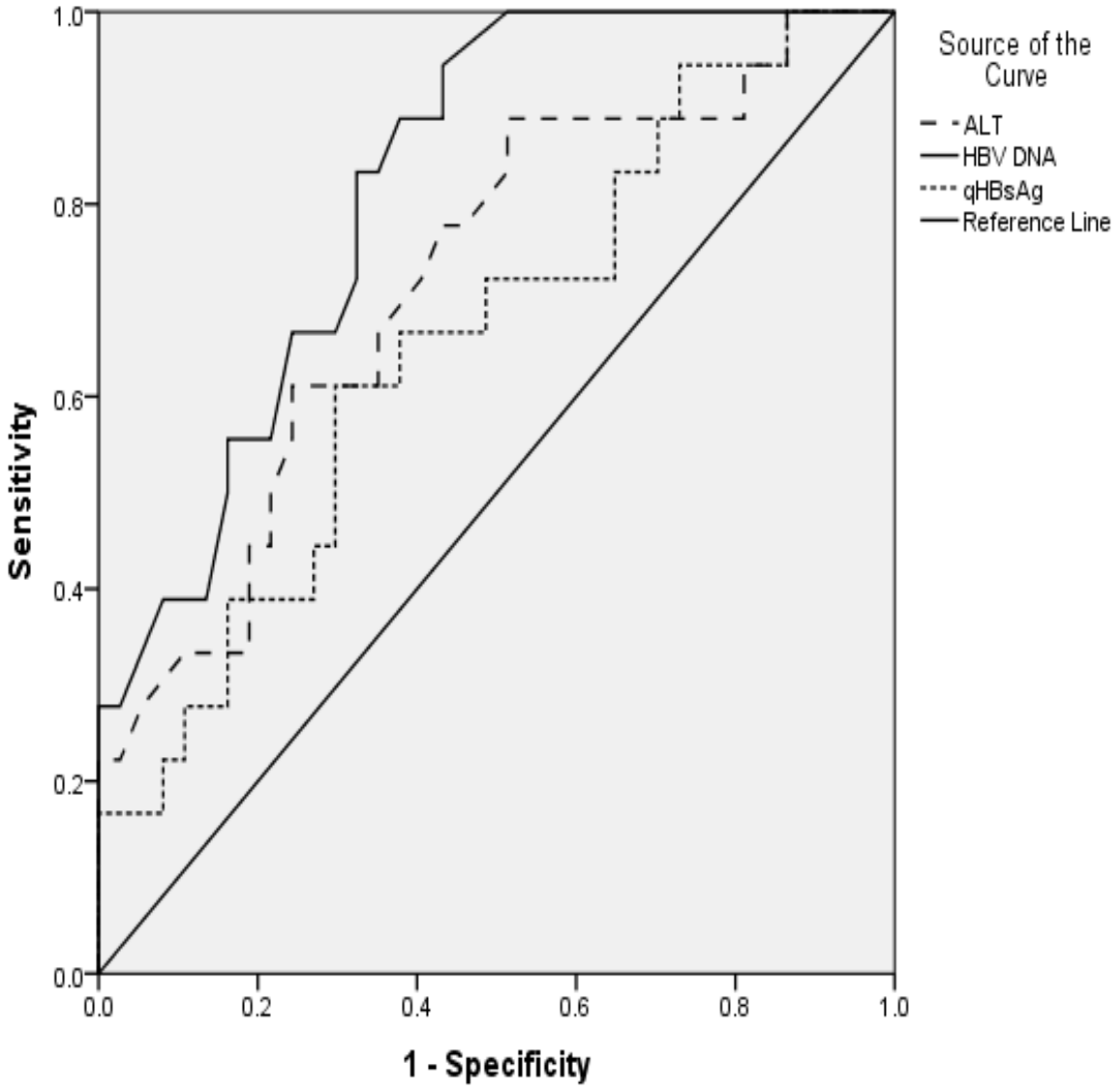

Fig 5: ROC curve for predicting CVR

ALT levels and correlation with qHBsAg:

Median ALT levels at baseline, 24 weeks, 48 weeks and 72 weeks were 41 (8 to 1518), 33.50 (9 to 574), 33(16 to 223) and 43 (30 to 86) respectively. In HBeAg + ve group median ALT levels were 46 (8 to 889), 32 (9 to 227), 33(16 to 223) and 53.5(42 to 86) respectively. In HBeAg – ve group median ALT levels were 31 (10 to 1518), 34.5 (10 to 574) , 31.5 (16 to 78),30.5(30 to 31).(See Fig 4).There was no correlation of ALT with qHBsAg at baseline, 24 weeks,48 weeks and 72 weeks. There was no significant difference in distribution of ALT levels across difference time points.

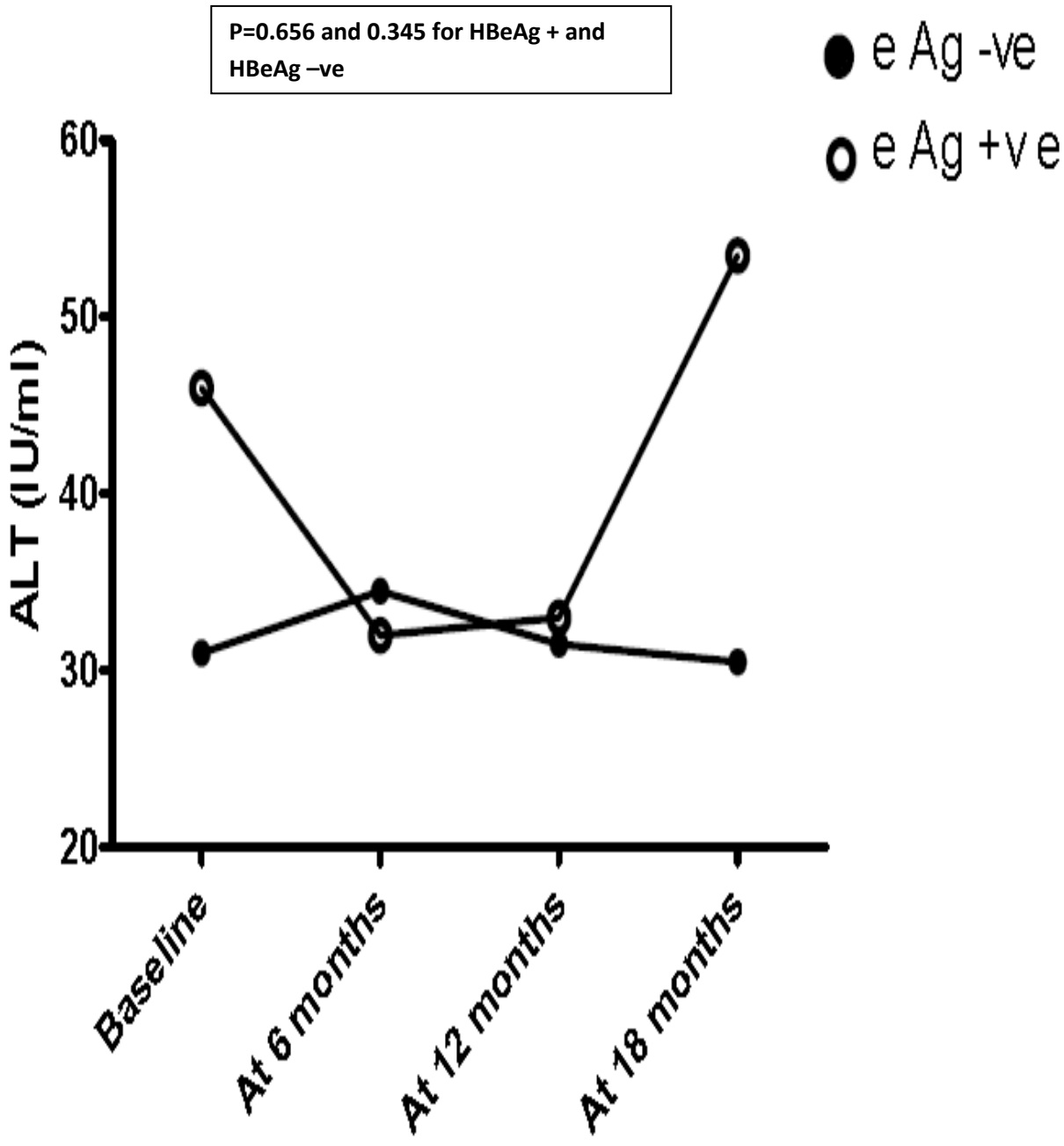

Fig4. ALT levels in HBeAg + and HBeAg - patients

Interferon Therapy:

Eight patients were started on interferon therapy. One patient received therapy for 48 weeks and he had e antigen seroconversion. Seven patients had failed interferon therapy and received therapy for atleast 6 months. qHBsAg and HBV DNA trends in these patients are depicted in fig 5. Median qHBsAg and HBV DNA at baseline,12 weeks,24 weeks and 48 weeks on IFN therapy was $\log_{10}$ 3.91 and $\log_{10}$ 6.04 , $\log_{10}$ 4.41 and $\log_{10}$ 5.69 , $\log_{10}$ 3.40 and $\log_{10}$ 4.77 and $\log_{10}$ 3.82 and $\log_{10}$ 5.95 respectively. qHBsAg and HBV DNA decline patterns in patient who responded to interferon therapy are given in Fig 6

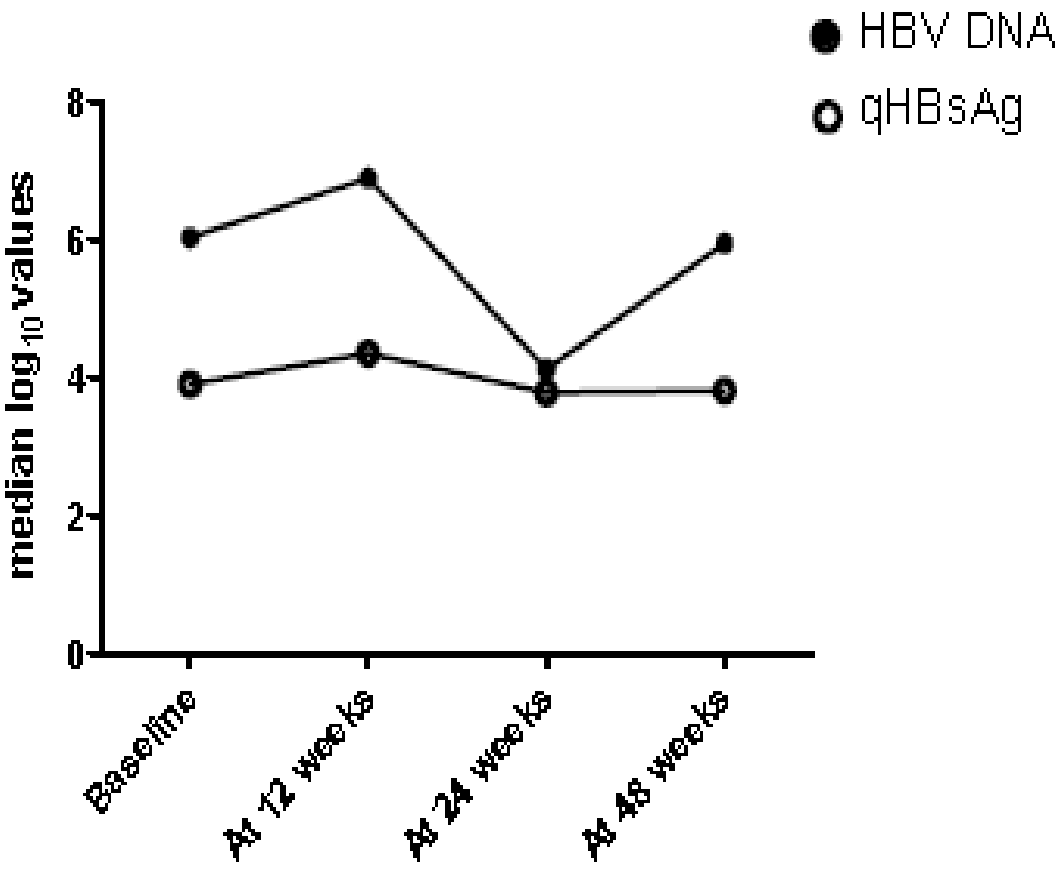

Fig 5: **qHBsAg and HBV DNA trends on interferon therapy**

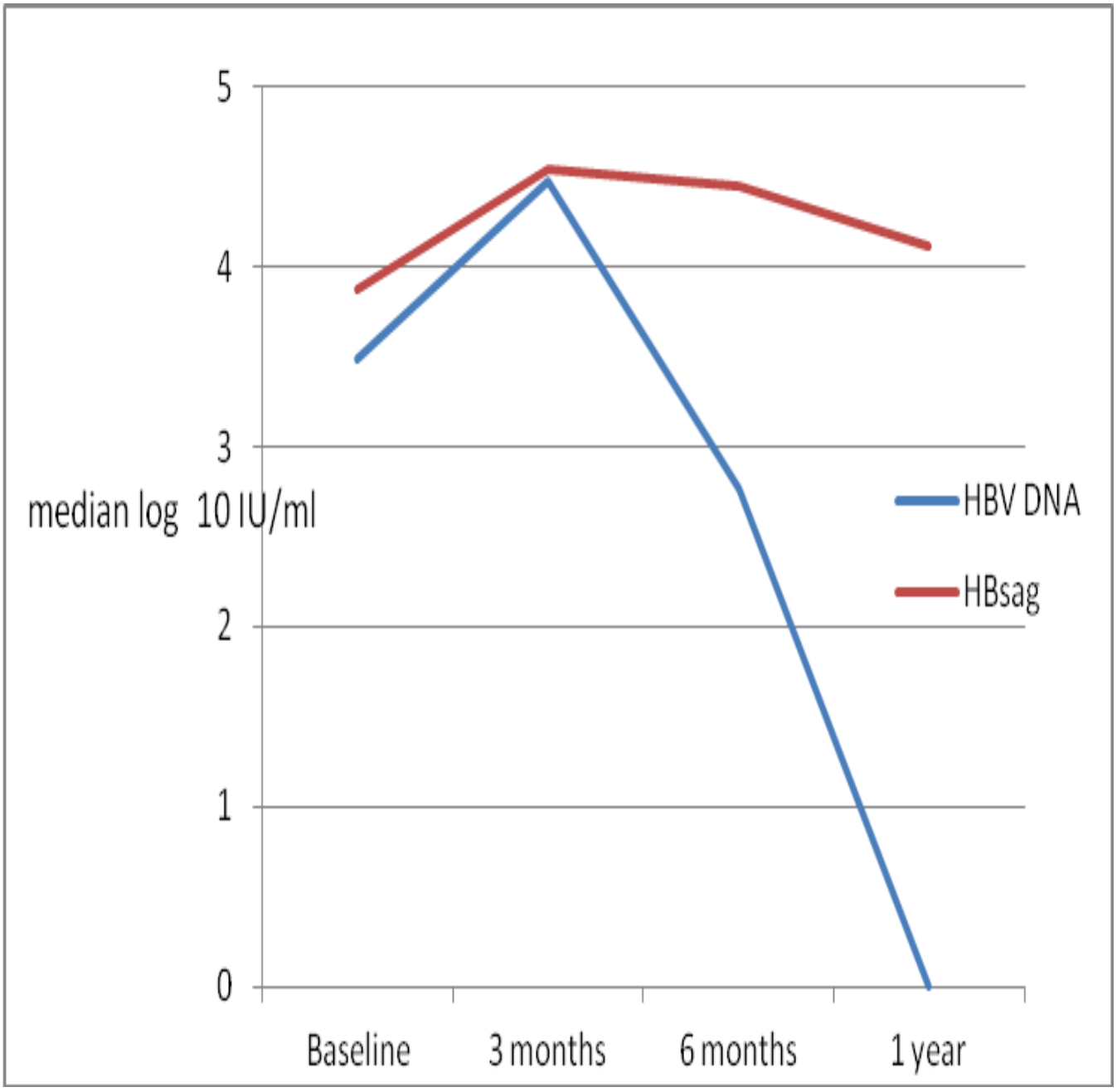

Fig 6: **qHBsAg and HBV DNA trend on successfully treated interferon therapy patient who achieved seroconversion**

DISCUSSION

Availability of quantitative HBsAg assays has renewed the interests of hepatologists worldwide. Many studies involving subjects from various ethnicities have been done. It is accepted that qHBsAg tends to be higher in patients who are HBeAg positive and low replicators can be identified with qHBsAg <1000 IU/ml (20) in genotype D patients. The data on qHBsAg dynamics on NA treatment is scarce and variable. Our aim was to look at the patterns of decline in qHBsAg level during antiviral therapy and its correlation with HBV DNA levels.

The clinical utility of qHBsAg in IFN treated patients is accepted and variable reductions have been shown to predict response to IFN. More reliably, non responders can be identified as early as 12 weeks, with lack of qHBsAg reduction (37,41,42). In our study 7/8 patients treated with IFN did not show any virological response (neither end of treatment nor sustained). In these patients, there was no decrease of qHBsAg was seen at any time point throughout the treatment. In fact, the levels increased before reaching the baseline value. In the single patient who responded also, the qHBsAg levels showed no reduction at all time points. Thus our study clearly shows that monitoring qHBsAg is useful in predicting non response to IFN. It is difficult to explain why the single responder also did not show any reduction in qHBsAg. Some studies have also looked at the baseline level of qHBsAg < 10,000 IU/L in predicting response to IFN (42). Our IFN responder did not have different baseline qHBsAg compared to non responders. Baseline qHBsAg level in patients who responded and non responders were $\log_{10}$ 3.96 IU/ml and 3.87 IU/ml respectively.

Though the exact decline of qHBsAg is not yet agreed upon, any fall in qHBsAg at 12 weeks is considered an excellent milestone for deciding on continuation of a costly therapy with major side effects. Our study also is in agreement with these observations (37,41,42), - though our numbers are very small. Unlike the western country, in India patients have to pay for their own treatment and decision of discontinuation early on makes a major impact on the willingness to even consider this potentially useful but expensive therapy.

In contrast clinical usefulness of qHBsAg in patients receiving oral NA therapy had been studied less extensively and results are not reproducible among different studies (27,28,31). There is no published information in this regard from India. Therefore data in this study becomes important as it looked at qHBsAg decline with potent antiviral drugs such as ETV and TDF as well with interferon therapy.

In the case of ENH, where oral NA is preferentially used, the end point of therapy is HBsAg clearance. The utility of qHBsAg is mainly to predict the durability of viral response once the NA are stopped and to predict who can actually be weaned off NA .Patients who had a steep fall in qHBsAg commensurate with DNA levels were most likely to achieve e antigen or S antigen clearance subsequently. This has been shown with telbivudine and Tenofovir therapy (30, 31). Earlier studies also showed a preferential reduction in qHBsAg levels in e Ag +ve patients (39, 47)

In HBeAg positive LdT treated patients, low qHBsAg levels at 2 years of treatment (<100 IU/ml) predicted SR for 2 years after stopping therapy (31).

In contrast in HBeAg –ve pts, treated with tenofovir or entecavir, the decline in qHBsAg is not that robust. The early decline has not been shown to predict long term surface antigen loss in Germans (31) while reduction in qHBsAg among LAM users from HongKong could predict sustained viral responders (36)

In our study no significant decline in qHBsAg was seen despite 70 % patients showing at least 2 log reduction in DNA levels. The qHBsAg levels were not reflective of presence of any viral response, complete response or lack of viral response. This leads to the conclusion that upto a follow up of 18 months, there are no changes in the hepatocytic burden of transcriptionally active HBV virions.

The major question in the minds of physicians and patients alike is the duration of NA treatment. At present, qHBsAg seems to be the only test which can predict who can be weaned off with a reasonable chance of success. Our study tells that atleast for a period of 18 months of antiviral therapy, no patient either Eag +ve or –ve showed any sign reduction in qHBsAg. No consideration of cessation of NA can be considered until a trend in the reduction of qHBsAg can be shown. Our study reinforces the transient nature of NA induced DNA suppression. Though it reduces the inflammatory and fibrotic changes in the liver, it doesn’t change the natural history of viral kinetics in the body.

Till qHBsAg reduction is demonstrated, it is clear that the immunological control of the disease hasn't begun and one needs to continue the NA therapy with the same vigour as in the beginning of treatment.

Advance in the quantification of qHBsAg has opened a new path for further understanding of chronic HBV infection. qHBsAg is known to reflect ccc DNA, which is the viral template for HBV replication in maintenance of chronic infection. The correlation between these two markers had been studied previously (10, 11).In our study also qHBsAg significantly correlated with HBV DNA(r =0.487) at baseline. Recent investigations have alluded to a potentially interesting aspect of qHBsAg as antigen expression in the natural course of HBV infection. Two independent groups found that the level of qHBsAg was higher in the immune-tolerant and immune-clearance phases than in the low-replicative phase and in patients with HBeAg(-) disease(14,19).In HBeAg(+) patients, significant correlation was noted (r ¼ 0.69, P < 0.001), whereas the association between HBsAg production and HBV replication broke down in HBeAg(-)patients (r ¼ 0.28, P ¼ 0.012)(15). These reports suggest that the correlation between qHBsAg and HBV DNA without antiviral treatment is more significant in the higher HBV replicative phase than in the low-replicative phase. In our study it correlated with HBV DNA in HBeAg + (r= 0.560) patients as opposed to HBeAg –ve patients (r=0.256) in agreement with other studies at baseline. At 6 months there was a weak correlation noted in HBeAg +ve patients(r=0.427) as opposed to HBeAg –ve patients(r=0.146).In HBeAg –ve patients there was correlation noted only after 18 months of therapy (r=0.894) but numbers were small(five patients).The correlation noted in HBeAg –ve patients is in contrast to

previous studies done in Hong Kong and Korea involving genotypes B and C.(27,28).The possible explanation for this finding may be related to genotypic differences .We are not sure of the clinical significance of this result. Whether it predicts a surface antigen clearance remains to be seen. Among different therapies, r value (correlation) progressively increased in ETV and IFN therapy whereas it decreased on TDF therapy (see Table 3). Median difference of qHBsAg at 48 weeks with ETV and TDF was not significant ($\log_{10}$ 0.27 and $\log_{10}$ 0.10 respectively). There are few explanations for this modest decline of qHBsAg .The possible explanation for this modest decline is related to mechanism of action of oral NA which is the suppression of viral replication through inhibition of HBV polymerase; because HBsAg production proceeds by a pathway distinct from that of HBV DNA, the effect of NA on qHBsAg is possibly less prominent.

A possible explanation for this lack of correlation between qHBsAg and HBV DNA is that the proportion of patients with undetectable or lower HBV DNA levels increased with antiviral therapy. Moreover, a modest decline of qHBsAg during ETV and TDF therapy as compared with the rapid reduction of HBV DNA was noted, and the result was the disproportional status of these two parameters.

HBV DNA and ALT were predictors of complete virological response (CVR) on univariate analysis ($p<0.05$) as compared to qHBsAg ($p=0.057$).On multivariate analysis HBV DNA was only predictor of CVR. Difference in qHBsAg (Δ qHBsAg) at 24 weeks was $\log_{10}$ 0.048 IU/ml and Δ qHBsAg in

HBeAg +ve patients was $\log_{10}$ 0.23 and Δ qHBsAg in HBeAg –ve patients was $\log_{10}$ 0 .042.HBV DNA,ALT and qHBsAg were not predictors of partial virological response (VR).

There were some limitations of our study. Firstly, genotypic analysis was not done. Previous studies done from our centre demonstrated predominance of genotype A and D, and thus our results would be applicable to genotype A and D patients (5). Second limitation of this study was that the follow-up period was short and none of our subjects had loss of HBsAg or HBeAg. Therefore, the predictive value of quantitative HBsAg levels for treatment induced HBsAg seroconversion cannot be evaluated.

The majority of CHB patients on antiviral therapy in our study did not show a significant decline in HBsAg levels despite HBV DNA suppression at 18 months but the trend needs to be observed over long term. This is important to predict the possibility of eventual cessation of NA, as it is a big financial burden for many of our patients.

Overall we found negligible reduction in qHBsAg levels as compared to DNA with NA consistent with what is described in literature. This reiterates the fact that HBsAg loss is not very common in patients on NA therapy and therapy probably needs to be continued lifelong. Since decline in qHBsAg may predict sustained viral response, it is suggested that this parameter be monitored along with DNA levels to look for the emergence of immune control of the disease.

CONCLUSIONS

1. Absence of any reduction in qHBsAg levels was associated with absence of response to IFN.
2. There was no significant reduction in qHBsAg levels after 18 months of NA therapy despite significant reduction in DNA levels.
3. Baseline HBV DNA level and not qHBsAg level was the only predictor of complete virological response.
4. No meaningful correlation was noted between qHBsAg and HBV DNA levels with either ETV or with TDF therapy. There was no correlation noted between ALT level and qHBsAg level during antiviral therapy.
5. The trend of qHBsAg levels needs to be monitored long term along with DNA levels while on NA therapy so that eventual sustained viral response after NA cessation can be predicted.

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Name	HospitalNo	Age	Sex	Eantigen	AST	ALT
MD Abul Kashem	043589f	45	M	NEGATIVE	30	16
Amitabh Chakrabo	617163d	58	M	NEGATIVE	41	31
Denis Chandra	965108d	28	M	NEGATIVE	27	26
Banladea	819112d	41	M	NEGATIVE	26	24
Nagachandra Moul	041154f	42	M	NEGATIVE	23	22
Sadanand Biswas	928955D	30	M	POSITIVE	61	63
Kajal kumar Bhat	890454d	39	M	POSITIVE	38	43
Biswajeet Jana	025434 f	19	M	NEGATIVE	47	55
Amarkant Das	991007d	46	M	NEGATIVE	23	18
Monoranjan Sarka	023357 F	37	M	NEGATIVE	75	39
Mohammed jamil	934272 d	24	M	POSITIVE	42	34
Ajit Kumar sarda	886295 d	39	M	NEGATIVE	46	59
Uttam Kumar Paul	761307d	48	M	NEGATIVE	48	92
Anil Kumar	990711D	29	M	NEGATIVE	33	45
Mahendra kumar y	009732f	41	M	NEGATIVE	70	96
Niraj Kumar	095502f	24	M	POSITIVE	45	37
Abhijit Jana	025428f	17	M	POSITIVE	37	48
Ashish Ranjan Si	048166f	30	M	POSITIVE	48	62
Akhilanand Singh	047339f	42	M	POSITIVE	47	35
Jitendra nath ma	970627d	41	M	POSITIVE	104	159
Debasis C	075593F	63	M	POSITIVE	35	25
Ajudhia Nath	433743c	67	M	NEGATIVE	94	33
Chelo tetup	986801D	62	M	POSITIVE	62	47
Moten Naiding	871750d	31	M	POSITIVE	81	38
Janaranjan Saha	727929d	55	M	NEGATIVE	27	23
Sudhangshu Roy	937846d	69	M	NEGATIVE	86	55
MDharoonrashid	104557f	40	M	NEGATIVE	29	22
Lalchhantluanga	592888 d	54	M	NEGATIVE	22	24
Biri Takar	863531d	21	M	NEGATIVE	27	16
Sreenivasa Mohan	888620 d	39	M	NEGATIVE	1,204	1,518
Kuppusamy	923126b	37	M	NEGATIVE	21	24
Chandra sekar d	915118d	26	M	POSITIVE	33	44
Venkata Ramaniah	050920f	35	M	NEGATIVE	31	50
SaBIHa Perween	004998f	29	F	POSITIVE	37	46
Gopinath Maji	916150d	20	M	POSITIVE	151	170
Chandra Bushan Y	911189d	30	M	POSITIVE	39	46
Ratan Kundu	062284f	32	M	POSITIVE	55	49
Keshav kumar Cho	579007d	21	M	POSITIVE	39	91
Jaba Saha	952893d	23	M	POSITIVE	121	140
Ata Ravi	048351 f	25	M	POSITIVE	35	31
Banumathi	173823d	30	F	POSITIVE	59	37
Sita Rani	023133F	48	F	NEGATIVE	86	61
Sunil Bhattachar	120624f	48	M	NEGATIVE	52	13
Kartik Paul	912028D	59	M	NEGATIVE	95	144
Kajol Mondal	017830F	7	M	POSITIVE	373	376
Madhu Sudan	026071d	60	M	NEGATIVE	23	10
Vishweshwaran	985445d	26	M	NEGATIVE	9	14
Sharunnappa Awaj	268894D	42	M	NEGATIVE	18	18
Sachu C	289512D	34	F	POSITIVE	34	32
Mohoammad Kama	042348F	34	M	NEGATIVE	32	29
Farida Yasmin	106098f	37	F	NEGATIVE	25	23

Mohammad Saiful	037443f	18	M	POSITIVE	30	34
Madhusudan purka	819147d	28	M	POSITIVE	30	52
Sankar Rana	973728d	41	M	POSITIVE	71	43
Bipin Bihari Raj	024510f	39	M	POSITIVE	39	43
Nayan Paul	029480f	31	M	NEGATIVE	71	#NULL!
Mantulal Hazra	850079D	36	M	NEGATIVE	30	23
Praneet Ansal Mu	952797d	26	M	NEGATIVE	326	574
Parimal Podder	867086d	53	M	NEGATIVE	107	68
Samar Kumar Bose	877576d	50	M	NEGATIVE	33	35
Sujit Karmakar	093986f	36	M	NEGATIVE	25	32
Badal Dhali	826156d	46	M	NEGATIVE	69	31
Abinash das	997425D	53	M	POSITIVE	128	57
Bidesh kumar man	134072f	30	M	POSITIVE	248	93
Revathi E	102884f	28	F	POSITIVE	16	8
Sanat kumar bata	973674d	35	M	POSITIVE	42	46
Bablu Mondal	774606d	26	M	POSITIVE	46	65
Nabadip Shaw	062133f	37	M	POSITIVE	50	41
Goutam Kumar Bar	925886d	33	M	POSITIVE	500	889
Manoj Kumar	936810d	44	M	NEGATIVE	25	20
Aditya kumar s	862504d	52	M	POSITIVE	355	115
Biswanath Pradha	016488f	26	M	POSITIVE	31	46
Bhanu biswas	982846 D	26	M	POSITIVE	25	27
Tamar Baki	994422d	33	M	NEGATIVE	35	30
Malamazumdar Da	957701d	33	F	POSITIVE	976	121
Guraja anand rao	713782c	49	M	POSITIVE	31	47
Muruganantham K	073857F	20	M	NEGATIVE	35	64
Sagila K	981330C	43	F	NEGATIVE	22	23
Mayil Vaganan	108935F	56	M	NEGATIVE	81	42
Subramaniyan P	137957f	52	M	NEGATIVE	56	42
Subramani V	083006F	41	M	NEGATIVE	25	41
Edi Mannan M	089521f	36	M	NEGATIVE	65	12
David r	822251 d	40	M	NEGATIVE	46	34
Muthu Kanan	791698c	66	M	NEGATIVE	114	78
Padam Priya	052650 C	33	F	NEGATIVE	25	38
Jeya kumar	564871a	53	M	NEGATIVE	31	22
Yogesh S	550718c	12	M	POSITIVE	39	39
Nandhini b	964713 d	19	F	POSITIVE	28	39

DNA0	log10	DNA1	log	DNA2	LOG2	DNA3	LOG3
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5,000	3.70	10	1.00	0	1.00	#NULL!	#NULL!
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100,000	5.00	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
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300,000,000	8.48	4,000	3.60	50	1.70	#NULL!	#NULL!
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50,000	4.70	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
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31	1.49	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
60,000	4.78	10	1.00	0	1.00	10	1.00
10,000,000	7.00	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
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60,000,000	7.78	8,000,000	6.90	1,000,000	6.00	10,000,000	7.00
4,000,000	6.60	10,000,000	7.00	60,000	4.78	900,000	5.95
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4,000,000	6.60	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
4,000	3.60	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
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57	1.76	0	1.00	#NULL!	#NULL!	#NULL!	#NULL!
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2,000,000	6.30	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
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30,000,000	7.48	483	2.68	140	2.15	#NULL!	#NULL!
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268	2.43	41	1.61	10	1.00	#NULL!	#NULL!
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2,000	3.30	68	1.83	#NULL!	#NULL!	#NULL!	#NULL!
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10,000	4.00	16	1.20	#NULL!	#NULL!	#NULL!	#NULL!
500,000,000	8.70	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
2,000,000	6.30	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
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242	2.38	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
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500,000	5.70	117	2.07	31	1.49	#NULL!	#NULL!
3,000	3.48	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
10,000	4.00	100,000	5.00	10	1.00	0	1.00
##########	9.00	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
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2,000	3.30	0	1.00	#NULL!	#NULL!	#NULL!	#NULL!
43	1.63	0	1.00	#NULL!	#NULL!	#NULL!	#NULL!
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147	2.17	0	1.00	10	1.00	#NULL!	#NULL!
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70,000,000	7.85	10,000	4.00	#NULL!	#NULL!	#NULL!	#NULL!

SAG0	LO0	SAG1	LO1	SAG2	LO2	SAG3	LO3
6,734	3.83	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
16,410	4.22	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
874	2.94	1,268	3.10	#NULL!	#NULL!	#NULL!	#NULL!
2,757	3.44	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
20,883	4.32	18,919	4.28	#NULL!	#NULL!	#NULL!	#NULL!
3,637	3.56	4,304	3.63	3,028	3.48	#NULL!	#NULL!
3,300	3.52	6,130	3.79	2,880	3.46	#NULL!	#NULL!
5,063	3.70	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
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437	2.64	710	2.85	651	2.81	#NULL!	#NULL!
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2,410	3.38	102	2.01	2,207	3.34	#NULL!	#NULL!
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88,379	4.95	110,818	5.04	50,039	4.70	#NULL!	#NULL!
250,000	5.40	57,739	4.76	#NULL!	#NULL!	#NULL!	#NULL!
54,903	4.74	28,841	4.46	40,887	4.61	#NULL!	#NULL!
173,345	5.24	110,511	5.04	#NULL!	#NULL!	#NULL!	#NULL!
266	2.43	145	2.16	#NULL!	#NULL!	#NULL!	#NULL!
16,146	4.21	18,094	4.26	#NULL!	#NULL!	#NULL!	#NULL!
7,344	3.87	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
1,954	3.29	952	2.98	667	2.82	956	2.98
1,797	3.25	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
2,803	3.45	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
13,198	4.12	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
1,804	3.26	844	2.93	1,501	3.18	1,737	3.24
21,297	4.33	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
426	2.63	231	2.36	1,456	3.16	#NULL!	#NULL!
60,704	4.78	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
23,208	4.37	19,831	4.30	#NULL!	#NULL!	#NULL!	#NULL!
31,265	4.50	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
8,778	3.94	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
250,000	5.40	23,134	4.36	1,941	3.29	6,616	3.82
2,464	3.39	7,427	3.87	2,517	3.40	2,006	3.30
7,392	3.87	34,834	4.54	28,029	4.45	12,842	4.11
7,287	3.86	28,845	4.46	14,923	4.17	#NULL!	#NULL!
949	2.98	34,834	4.54	2,006	3.13	#NULL!	#NULL!
24,929	4.40	22,831	4.36	#NULL!	#NULL!	#NULL!	#NULL!
98	1.99	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
9,373	3.97	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
1,374	3.14	4,053	3.61	#NULL!	#NULL!	#NULL!	#NULL!
40,082	4.60	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
25	1.40	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
5,232	3.72	3,811	3.58	#NULL!	#NULL!	#NULL!	#NULL!
33,633	4.53	44,190	4.65	#NULL!	#NULL!	#NULL!	#NULL!
69,268	4.84	104,239	5.02	#NULL!	#NULL!	#NULL!	#NULL!
60,058	4.78	83,662	4.92	#NULL!	#NULL!	#NULL!	#NULL!
23,520	4.37	33,994	4.53	#NULL!	#NULL!	#NULL!	#NULL!

32,222	4.51	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
10,935	4.04	14,435	4.16	14,605	4.16	17,106	4.23
34,788	4.54	124	2.09	2	0.26	#NULL!	#NULL!
5,532	3.74	5,452	3.74	#NULL!	#NULL!	#NULL!	#NULL!
2,343	3.37	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
8,654	3.94	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
23,338	4.37	49,078	4.69	44,246	4.65	#NULL!	#NULL!
3,117	3.49	2	0.35	3	0.41	#NULL!	#NULL!
1,966	3.29	2,814	3.45	#NULL!	#NULL!	#NULL!	#NULL!
23,991	4.38	28,629	4.46	#NULL!	#NULL!	#NULL!	#NULL!
3,605	3.56	2,114	3.33	#NULL!	#NULL!	#NULL!	#NULL!
924	2.97	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
1,925	3.28	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
47,914	4.68	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
82,065	4.91	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
19,860	4.30	19,454	4.29	20,387	4.31	#NULL!	#NULL!
6,930	3.84	2,008	3.30	#NULL!	#NULL!	#NULL!	#NULL!
250,000	5.40	60,462	4.78	#NULL!	#NULL!	#NULL!	#NULL!
11,128	4.05	24,377	4.39	#NULL!	#NULL!	#NULL!	#NULL!
9,631	3.98	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
10,777	4.03	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
114,350	5.06	40,282	4.61	#NULL!	#NULL!	#NULL!	#NULL!
2,583	3.41	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
125,000	5.10	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
13,135	4.12	17,293	4.24	8,815	3.95	#NULL!	#NULL!
38,403	4.58	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
858	2.93	1,298	3.11	1,236	3.09	1,109	3.04
116,818	5.07	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
14,524	4.16	21,269	4.33	11,472	4.06	11,472	4.06
3	0.41	3	0.42	#NULL!	#NULL!	#NULL!	#NULL!
2	0.19	2	0.19	#NULL!	#NULL!	#NULL!	#NULL!
24,886	4.40	38,012	4.58	23,715	4.38	38,012	4.58
8,446	3.93	6,396	3.81	8,446	3.93	#NULL!	#NULL!
15,020	4.18	24,970	4.40	#NULL!	#NULL!	#NULL!	#NULL!
25,712	4.41	20,903	4.32	#NULL!	#NULL!	#NULL!	#NULL!
250,000	5.40	240,256	5.38	95,214	4.98	#NULL!	#NULL!
27,047	4.43	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!

USG	BIOPSY	CLINICAL	DRUG	VAR00001
0			ETV	1.00
0			ETV	1.00
0		ENH	ETV	1.00
0		CLD	ETV	1.00
1		ENH + CLD	ETV	1.00
1		IR	ETV	1.00
1		CLD	ETV	1.00
0			ETV	1.00
0			ETV	1.00
1		ENH + CLD	ETV	1.00
1			ETV	1.00
0		ENH	ETV	1.00
0		ENH	ETV	1.00
0		ENH + CLD	ETV	1.00
0			ETV	1.00
0			ETV	1.00
0			ETV	1.00
0		IR	ETV	1.00
0		IT	ETV	1.00
0		IR	ETV	1.00
0		IT	ETV	1.00
1		LR + CLD	ETV	1.00
1		IR = CLD	ETV	1.00
1			ETV	1.00
0		LR + CLD	ETV	1.00
1			ETV	1.00
1			ETV	1.00
1			ETV	1.00
1		ENH + CLD	ETV	1.00
0			ETV	1.00
0		IR	ETV	1.00
0			ETV	1.00
0		ENH	ETV	1.00
0			IFN	3.00
0			IFN	3.00
0		IR	IFN	3.00
0		IR	IFN	3.00
0		IR	IFN	3.00
0		IR	IFN	3.00
1			IFN	3.00
0		IT	IFN	3.00
0			LAM	#NULL!
1			LAM	#NULL!
1		ENH + CLD	LAM	#NULL!
0			LAM	#NULL!
0			LAM	#NULL!
0	mild steatosis with portal a lobularinfl	LR	LAM	#NULL!
0		LR	LAM	#NULL!
0		IT	LAM/ADF	#NULL!
0	HAI 4/18 MILD PORTALFIBROSIS 2/6	ENH	TDF	2.00
0		ENH	TDF	2.00

0			TDF	2.00
1		CLD	TDF	2.00
1		IR	TDF	2.00
1		IR + CLD	TDF	2.00
0			TDF	2.00
0			TDF	2.00
0		ENH	TDF	2.00
1			TDF	2.00
1		ENH	TDF	2.00
1			TDF	2.00
1		ENH + CLD	TDF	2.00
1			TDF	2.00
1			TDF	2.00
0			TDF	2.00
0			TDF	2.00
0			TDF	2.00
0		IR + CLS	TDF	2.00
0		IR	TDF	2.00
1			TDF	2.00
1			TDF	2.00
0			TDF	2.00
0		IT	TDF	2.00
1			TDF	2.00
0			TDF	2.00
1		IR + CLD	TDF	2.00
0			TDF	2.00
0	HAI-5/18, EARLY PORTAL FIBROSIS	ENH	TDF	2.00
1			TDF	2.00
1		ENH + CLD	TDF	2.00
1		ENH + CLD	TDF	2.00
1		LR + CLD	TDF	2.00
0		ENH	TDF	2.00
0			TDF	2.00
0		ENH	TDF	2.00
0		ENH	TDF	2.00
0		IT	TDF	2.00
0			TDF	2.00

remarks	AST1	ALT1	AST2	ALT2	COMPLIANC	RENALFAILUI	AST3
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	26	10	#NULL!	#NULL!			#NULL!
	23	26	#NULL!	#NULL!			#NULL!
	25	24	#NULL!	#NULL!			#NULL!
	105	143	58	79			#NULL!
	38	31	33	30			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	64	29	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	48	33	28			#NULL!
	36	57	27	25			#NULL!
	29	49	43	78			#NULL!
	57	47	29	18			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	29	31	24	32			#NULL!
	37	29	27	21			#NULL!
	81	143	24	16			#NULL!
	#NULL!	27	#NULL!	#NULL!			#NULL!
	27	14	#NULL!	#NULL!			#NULL!
	60	47	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	27	23	27	29			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	44	42	38	39			30
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	29	34	26	34		NO	#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	27	38	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	152	277	32	33	GOOD	NO	29
	52	75	47	33	GOOD	NO	#NULL!
	54	91	72	151	GOOD	NO	41
	#NULL!	9	#NULL!	223			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	34	#NULL!	#NULL!	POOR	NO	#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	144	95	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	24	14	#NULL!	#NULL!			#NULL!
ALL PT.	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	24	16	#NULL!	#NULL!			#NULL!
	18	12	#NULL!	#NULL!			#NULL!
	#NULL!	23	#NULL!	#NULL!			#NULL!

	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	28	31	35	46			44
	39	25	60	24			61
	36	45	#NULL!	#NULL!			#NULL!
	81	30	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
reac	326	574	50	56			#NULL!
	#NULL!	#NULL!	70	37			#NULL!
	41	35	#NULL!	#NULL!			#NULL!
	#NULL!	37	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	25	28	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	29	33	30	38			#NULL!
	63	50	#NULL!	#NULL!			#NULL!
	53	36	#NULL!	#NULL!			#NULL!
	29	35	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	28	20	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	37	23	29	16			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	60	98	22	16	GOOD	NO	#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	56	42	52	39			38
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	31	20	#NULL!	#NULL!			#NULL!
	41	51	#NULL!	27			#NULL!
	42	#NULL!	#NULL!	#NULL!			#NULL!
	19	20	#NULL!	#NULL!			#NULL!
	20	19	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!
	#NULL!	#NULL!	#NULL!	#NULL!			#NULL!

ALT3	a	d	Therapy	VR	EAg	vcr	Ethnicity
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	bangladesh
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	bangladesh
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	Bangladesh
#NULL!	5.00	2.17	#NULL!	1.00	0.00	0.00	Bangladesh
#NULL!	5.00	2.17	#NULL!	1.00	0.00	0.00	east
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	east
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	east
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	east
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	east
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
#NULL!	5.00	2.17	#NULL!	1.00	0.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	0.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	East
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	North
#NULL!	5.00	2.17	#NULL!	0.00	1.00	0.00	north east
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	North east
#NULL!	5.00	2.17	#NULL!	1.00	0.00	0.00	North east
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	North East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	North East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	North East
31	5.00	2.17	#NULL!	1.00	0.00	1.00	North East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	south
#NULL!	5.00	2.17	#NULL!	0.00	0.00	0.00	south
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	south
#NULL!	5.00	2.17	#NULL!	1.00	0.00	0.00	South
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
44	5.00	2.17	#NULL!	0.00	1.00	0.00	East
#NULL!	5.00	2.17	#NULL!	0.00	1.00	0.00	East
86	5.00	2.17	#NULL!	0.00	1.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	South
#NULL!	5.00	2.17	#NULL!	0.00	1.00	0.00	South
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	east
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	East
#NULL!	5.00	2.17	#NULL!	1.00	0.00	0.00	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	north east
#NULL!	5.00	2.17	#NULL!	0.00	0.00	1.00	south
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	South
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	South
#NULL!	5.00	2.17	#NULL!	0.00	0.00	0.00	Bangladesh
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	Bangladesh

#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	Bangladesh
63	5.00	2.17	#NULL!	1.00	1.00	0.00	east
42	5.00	2.17	#NULL!	1.00	1.00	0.00	east
#NULL!	5.00	2.17	#NULL!	1.00	1.00	1.00	east
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	east
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	east
#NULL!	5.00	2.17	#NULL!	0.00	0.00	0.00	east
#NULL!	5.00	2.17	#NULL!	0.00	0.00	0.00	East
#NULL!	5.00	2.17	#NULL!	0.00	0.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	East
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	East
#NULL!	5.00	2.17	#NULL!	0.00	1.00	0.00	East
#NULL!	5.00	2.17	#NULL!	0.00	1.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	East
#NULL!	5.00	2.17	#NULL!	1.00	0.00	0.00	North
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	North
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	North east
#NULL!	5.00	2.17	#NULL!	1.00	1.00	1.00	North east
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	North East
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	1.00	#NULL!	North East
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	south
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	south
#NULL!	5.00	2.17	#NULL!	0.00	0.00	0.00	south
#NULL!	#NULL!	#NULL!	#NULL!	#NULL!	0.00	#NULL!	South
30	5.00	2.17	#NULL!	0.00	0.00	0.00	South
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	South
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	South
#NULL!	5.00	2.17	#NULL!	1.00	0.00	0.00	South
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	South
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	South
#NULL!	5.00	2.17	#NULL!	1.00	0.00	1.00	South
#NULL!	5.00	2.17	#NULL!	1.00	1.00	0.00	South
#NULL!	#NULL!	#NULL!	#NULL!	1.00	1.00	0.00	South

Followup	nonresponse	filter_$
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	1.00	1
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	1.00	1
#NULL!	1.00	1
#NULL!	1.00	1
#NULL!	0.00	0
#NULL!	1.00	1
#NULL!	1.00	1
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	1.00	1
#NULL!	1.00	1
#NULL!	0.00	0
#NULL!	1.00	1
#NULL!	0.00	0

#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	1.00	1
#NULL!	1.00	1
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	#NULL!	#NULL!
#NULL!	1.00	1
#NULL!	#NULL!	#NULL!
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	1.00	1
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0
#NULL!	0.00	0